ATTACHMENT A

Exemplary Infringement of U.S. Patent No. 10,227,639 by NanoString's CosMx Spatial Molecular Imaging Platform and Workflow

Claim No.	Claim Language	NanoString Spatial Molecular Imaging
1 (Preamble)	A method for analyte identification, comprising:	To the extent that the preamble is found to be limiting, NanoString's CosMx Spatial Molecular Imaging ("CosMx SMI") workflow, including when used by NanoString through its CosMx Technology Access Program, is a method for analyte (RNA or protein) identification. "SMI is a completely automated and integrated platform comprising chemistry, hardware, and software that enables highly sensitive spatial profiling of 980 RNAs and 108 proteins in FFPE tissues at single-cell and subcellular resolution." "High-Plex Multiomic Analysis in FFPE at Single-Cellular and Subcellular Level by Spatial Molecular Imaging," Shanshan He *et al.*, (preprint available at https://www.biorxiv.org/content/10.1101/2021.11.03.467020v2) ("He-Beechem") at 4 "CosMx SMI is an integrated system with mature cyclic fluorescent in situ hybridization (FISH) chemistry, high-resolution imaging readout, and interactive data analysis and visualization software."

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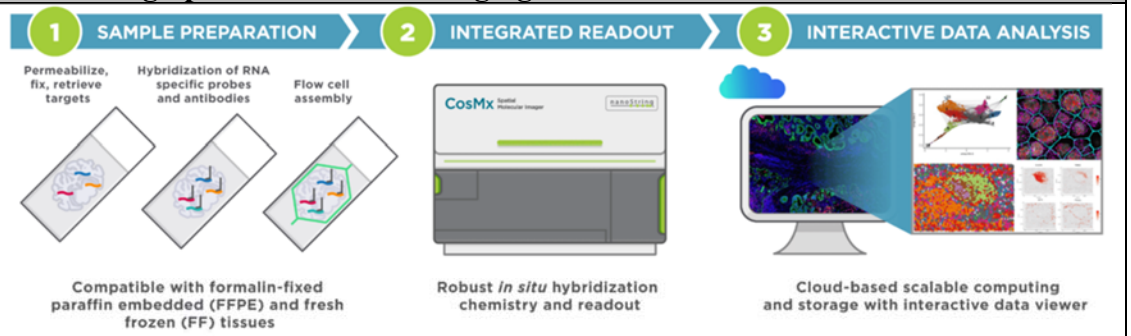
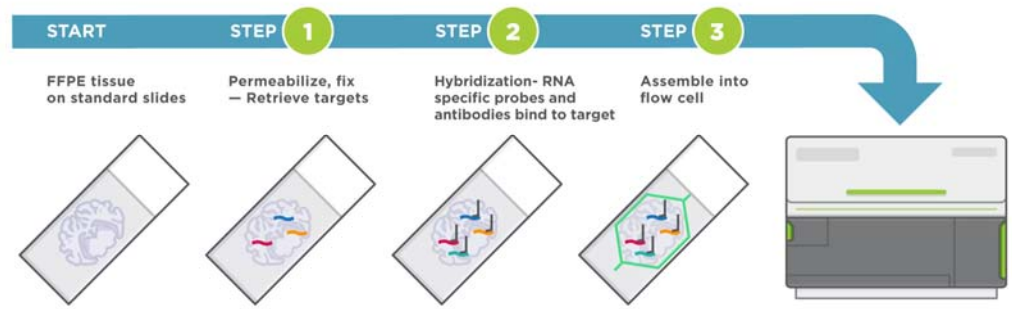
		 **Easy Sample Preparation, Compatible with Any Sample Type**  Streamlined and simple workflow that integrates with standard ISH protocol with no need for tissue expansion or clearing, cDNA synthesis or amplification. Go from sample to result faster.

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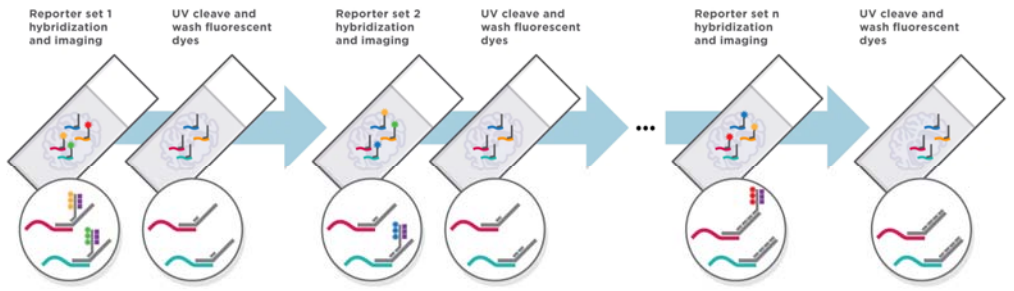
		Automated Cyclic *in situ* Hybridization Chemistry  Robust hybridization chemistry that provides higher sensitivity and supports high plex assays in your tissue samples to uncover deeper biological insights. "CosMx Spatial Molecular Imager," available at https://www.nanostring.com/products/cosmx-spatial-molecular-imager/overview/ ("CosMx SMI Overview") "NanoString provides complete spatial molecular imaging service through Technology Access Program (TAP) from our state-of-the-art laboratory. The Spatial Molecular Imaging TAP enables researchers to visualize and quantify up to 1,000 RNA expression directly in single cells with their spatial context preserved." "CosMx SMI Technology Access Program," https://www.nanostring.com/products/cosmx-spatial-molecular-imager/technology-access-program/ ("CosMx SMI TAP")

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		"How It Works: 01: Submit your Formalin-Fixed Paraffin-Embedded tissue samples (whole mount tissue sections or tissue microarrays) to NanoString's Translational Services Lab 02: Nanostring will analyze your samples using spatial molecular imaging system with up to 1,000-plex RNA panel. 03: Receive detailed report including raw data and analyzed results in 6-10 weeks." CosMx SMI TAP
1[a]	(a) contacting a sample with a plurality of detection reagents, wherein said plurality of detection reagents comprises a detection reagent that targets an analyte of a plurality of analytes immobilized in the sample, wherein said detection reagent comprises: (i) probe targeting said analyte and (ii) a nucleic acid label comprising a plurality of pre-determined subsequences,	NanoString's CosMx SMI workflow, including when performed by NanoString as part of its CosMx Technology Access Program, includes contacting a sample with a plurality of detection reagents, where the plurality of detection reagents comprises: a detection reagent that targets an analyte from among a plurality of analytes immobilized in the sample and includes a probe (RNA-specific probe or antibody probe) that targets the analyte (target RNA or protein, respectively) and that is conjugated together with a nucleic acid label comprising two or more of pre-determined subsequences comprising an identifier of the probe.

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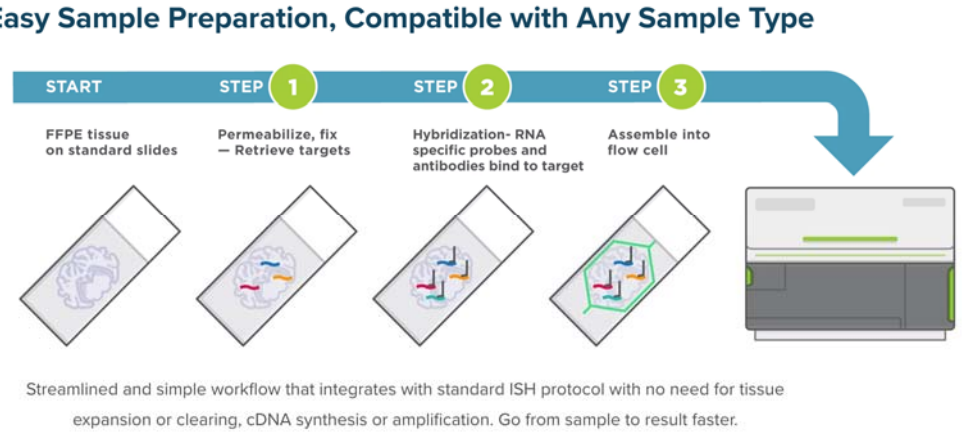
	wherein said probe and said nucleic acid label are conjugated together, and wherein said plurality of pre-determined subsequences forms an identifier of said probe;	 CosMx SMI Overview "For sample preparation, SMI utilizes standard methods performed for FISH on FFPE tissue sections to expose RNA targets, followed by the introduction of fluorescent bead-based fiducials that are fixed to the tissue to provide an optical reference for cyclic image acquisition and registration." He-Beechem at 5

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		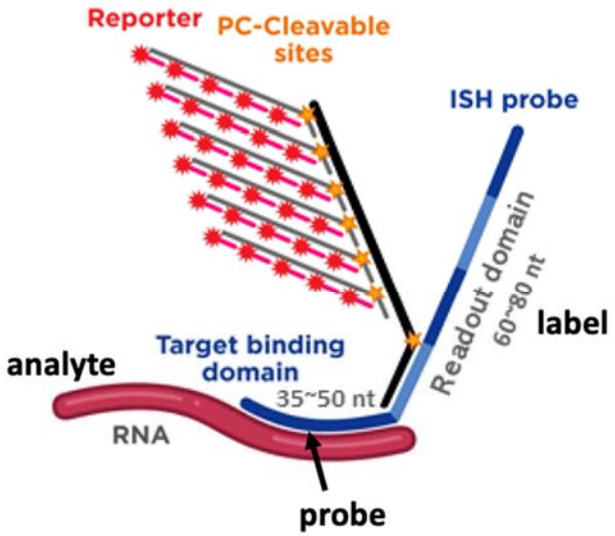 He-Beechem, Fig. 1A (annotated) "Schematic description of SMI ISH probe and reporter design. ISH probe consists of the **target-binding domain and readout domain. The target-binding domain is a 35-50 nt DNA sequence that hybridizes with target RNA. The readout domain is a 60-80 nt DNA sequence that contains 4 consecutive 10-20 nt reporter-landing domains, where each landing domain can be hybridized with a unique reporter.** With a 64-bit barcoding design, there are a total of 64 unique reporter-landing sequences." He-Beechem at 8, notes at Fig. 1A (emphasis added)

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		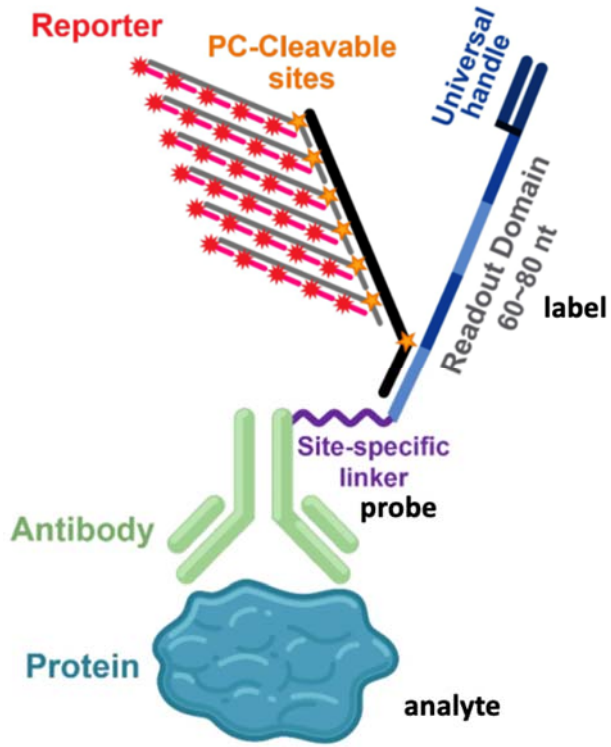 He-Beechem, Fig. S13 (annotated) "Protein Detection Probe. The protein detection probe is readily adapted from the RNA detection probe shown in Figure 1. For protein detection, the readout domain contains an additional universal handle and is conjugated to the antibody via a site-specific linker." He-Beechem at 53, notes at Fig. S13

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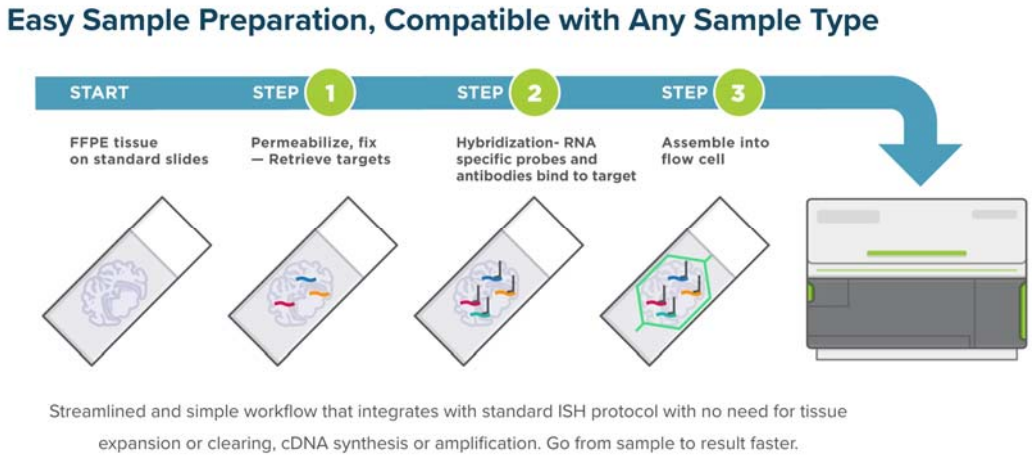
1[b]	(b) with said analyte immobilized in the sample and said probe coupled to said analyte, (i) hybridizing a first decoder probe with a first subsequence of said plurality of pre-determined subsequences, wherein said first decoder probe comprises a first detectable label, (ii) detecting a first signal signature from said first detectable label, (iii) hybridizing a second decoder probe with a second subsequence of said plurality of pre-determined subsequences, wherein said second decoder probe comprises a second detectable label, and (iv) detecting a second signal signature from said second detectable label, to provide a set of signal signatures comprising said	In NanoString's CosMx SMI workflow, including when performed by NanoString as part of its CosMx Technology Access Program, the analyte (RNA and/or protein) is immobilized in the sample and the probe (RNA-specific probe or antibody probe) is coupled (hybridized) to the analyte. A first decoder probe (reporter probe) comprising a first detectable (color) label is hybridized with a first subsequence of the label and a first signal signature is detected from the first detectable label. A second decoder probe comprising a second detectable label is hybridized with a second subsequence of the label and a second signal signature is detected from the second detectable label. A set of signal signatures is provided comprising the first signal signature and second signal signature.  CosMx SMI Overview "For sample preparation, SMI utilizes standard methods performed for FISH on FFPE tissue sections to expose RNA targets, followed by the introduction of

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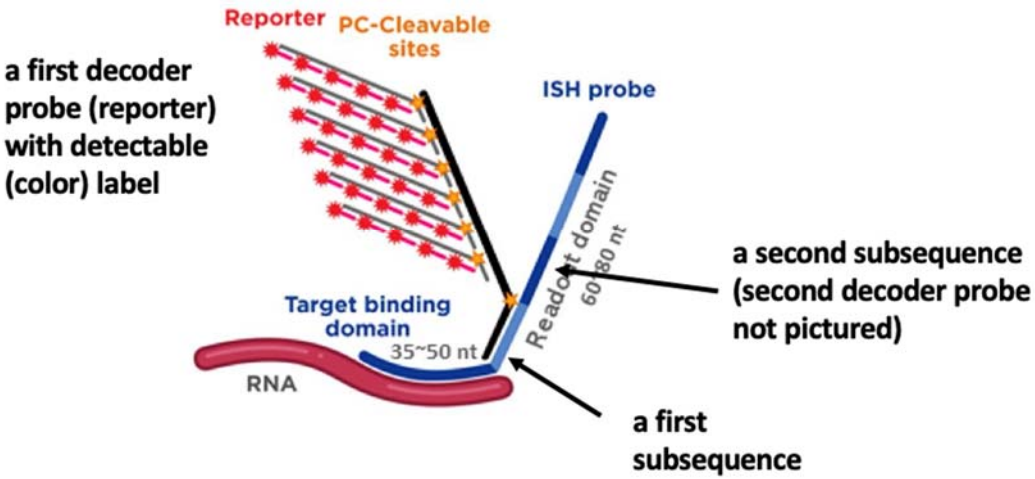
	first signal signature and said second signal signature; and	fluorescent bead-based fiducials that are fixed to the tissue to provide an optical reference for cyclic image acquisition and registration." He-Beechem at 5  He-Beechem, Fig. 1A (annotated) "Schematic description of SMI ISH probe and reporter design. ISH probe consists of the target-binding domain and readout domain. The target-binding domain is a 35-50 nt DNA sequence that hybridizes with target RNA. **The readout domain is a 60-80 nt DNA sequence that contains 4 consecutive 10-20 nt reporter-landing domains, where each landing domain can be hybridized with a unique reporter.** With a 64-bit barcoding design, there are a total of 64 unique reporter-landing sequences." He-Beechem at 8, notes at Fig. 1A (emphasis added)

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		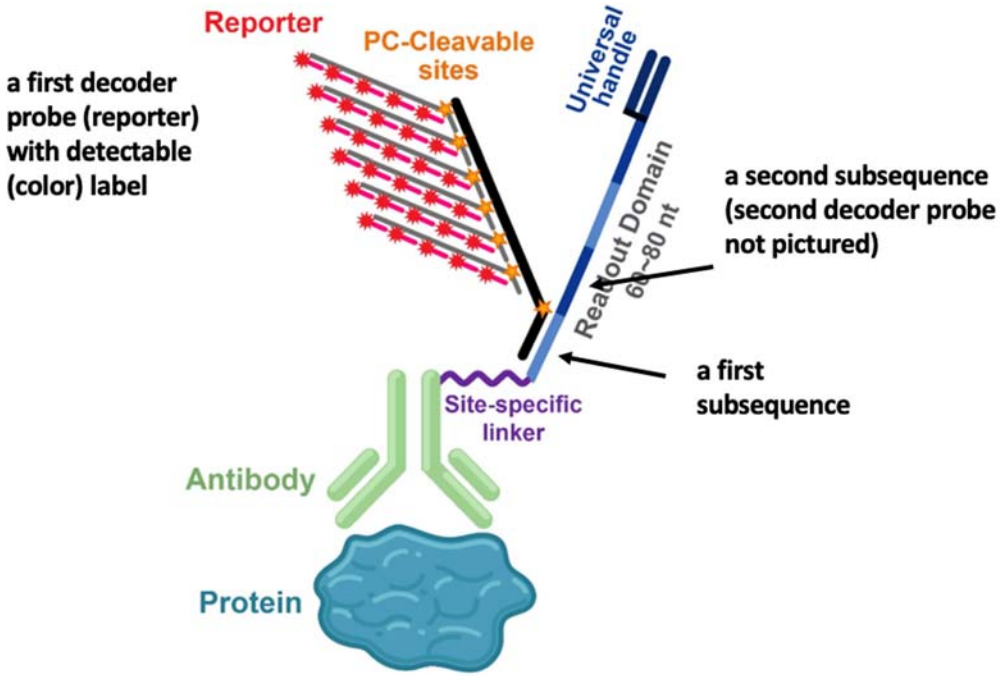 He-Beechem, Fig. S13 (annotated) "Protein Detection Probe**.** The protein detection probe is readily adapted from the RNA detection probe shown in Figure 1. For protein detection, the readout domain contains an additional universal handle and is conjugated to the antibody via a site-specific linker." He-Beechem at 53, notes at Fig. S13

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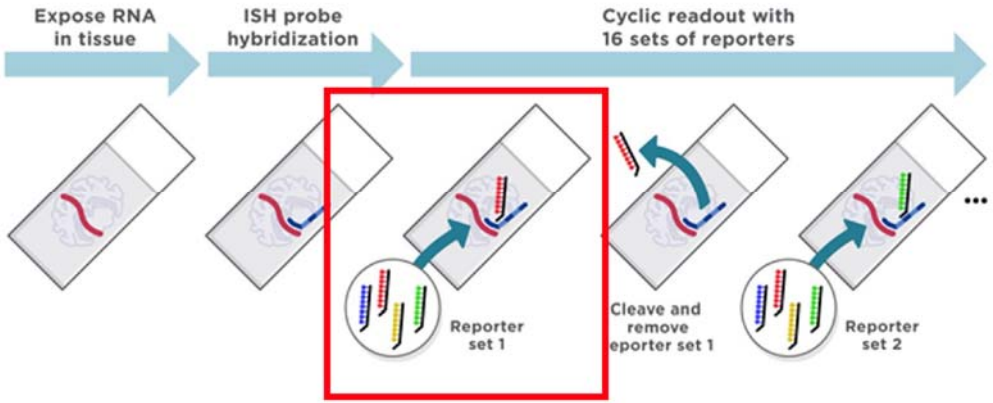
		"Following hybridization of ISH probes, slides are washed, assembled into a flow cell, and placed within a fluidic manifold on the SMI instrument for RNA readout and morphological imaging. **In RNA assay readout, the tissue is hybridized with 16 sets of fluorescent reporters sequentially; each reporter set contains four single-color reporter pools.** The reporters specifically bind to ISH probes during the 16 rounds of reporter hybridization according to the barcode assigned to each gene (Figure S1). After the incubation of each set of reporters, high-resolution Z-stacked images are acquired for downstream analysis." He-Beechem at 5 (emphasis added)  He-Beechem, Fig. 1B (annotated)

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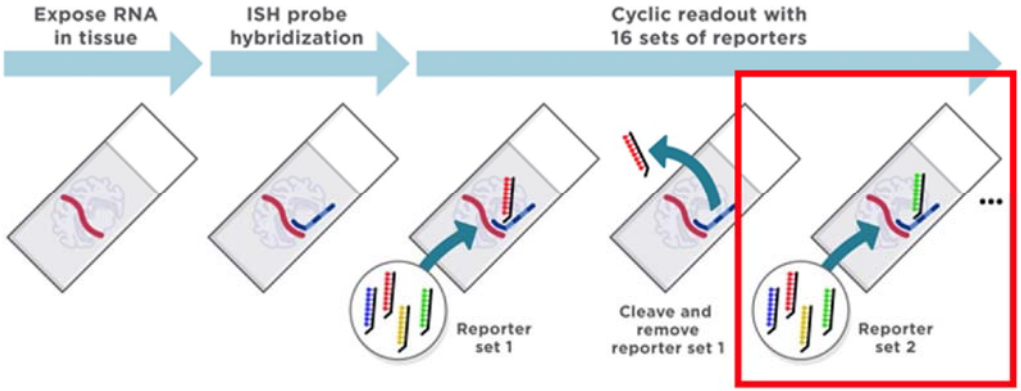

He-Beechem, Fig. 1B (annotated)

"SMI encoding scheme is designed to assign a unique barcode to each target transcript from a set of 64-bit barcodes (4 color reporters in each readout round over 16 readout rounds) with Hamming distance 4 (HD4) and Hamming weight 4 (HW4). Every barcode is separated by a Hamming distance of at least 4 from all other barcodes to maximally suppress RNA decoding error. Every barcode has a constant HW4, in which each target is 'on' in 4 rounds and 'off' in 12 rounds during the 16 rounds of reporter hybridization. This 'on' and 'off' signal barcode design allows for continued expansion to even higher plex since only a subset of RNAs is 'on' in any given cycle. Note that the nature of these (on-off) imaging signals represents a 'deterministic super-resolution imaging system', and hence each SMI imaging barcode can be located well below the diffraction limit of the imaging system. **For each reporter hybridization round, a single reporter can bind to one of the reporter-landing domains on the ISH probe of the gene (Figure 1A and B, Figure S1)**. The 64-bit encoding scheme with

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		the HD4 and HW4 yields 1,210 barcodes, from which a subset of 980 barcodes is selected to detect 960 target genes and 20 negative probe controls (Table S1)." He-Beechem at 5 (emphasis added) "The combination of SMI chemistry, hardware, and software enables high-plex spatial RNA profiling within single cells with very high sensitivity. For spatial profiling, the data of fluorescent signals in Z-stacked raw images is transformed into decoded RNA transcript information at their registered spatial location in three dimensions (3D) and assigned to single cells that are identified by segmentation (Figure 1C). **During the primary image processing, the fluorescence signals in raw images are transformed into digital output comprising of detected 'spots' localized in X-, Y-, and Z-dimensions for subsequent RNA decoding.** A spot is identified in an image as an isolated fluorescence signal with the intensity much higher than its neighbors. In biological tissues, however, dense fluorescent spots can be detected at a small spatial region, which may limit the accuracy of resolving each spot. To solve this potential issue, we developed custom image analysis algorithms to process 3D multi-channel image stacks obtained in each field of view (FOV). The key objective of this analytical method is to reduce the multi-dimensional image stack to a single list of individual reporter-binding events. This process is performed across all FOVs, concurrently with image acquisition during cyclic reporter readout. All spots pertaining to a given FOV are collated into a single list of XYZ locations of all individual reporter-binding events. This list is used in the next step to decode the gene-specific barcodes formed by these reporter-binding events." He-Beechem at 8-9 (emphasis added)
1[c]	(c) comparing said set of signal signatures against set of signal signatures assigned to different	In NanoString's CosMx SMI workflow, including when performed by NanoString as part of its CosMx Technology Access Program, the set of signal signatures are compared to a list of signal signatures for various analytes include the target analyte to identify the probe and thereby identify the analyte in the sample.

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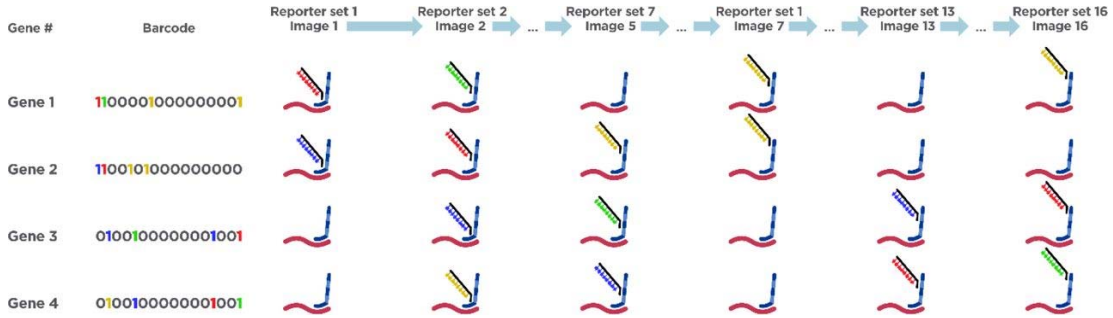
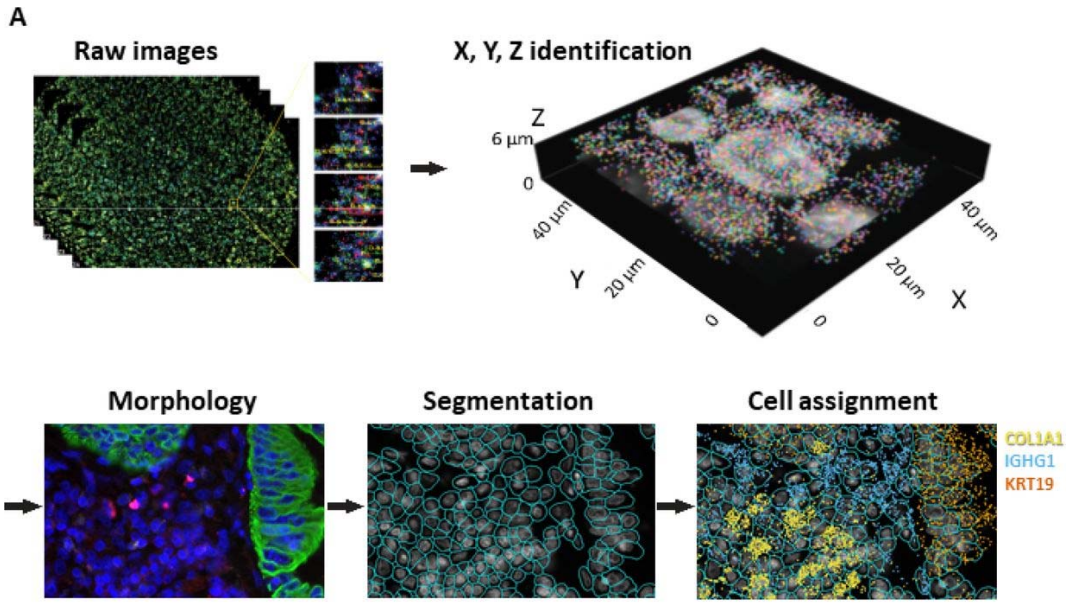
	analytes including said analyte, to identify said probe, thereby identifying said analyte immobilized in the sample.	"Each gene is assigned with a unique 16-digit barcode with 4 'on' spots (labeled as '1') and 12 'off' spots (labeled as '0'). Each digit of the barcode indicates the presence of the reporter that is associated with the target in the specific reporter hybridization round. '1' means that there is a reporter hybridizing to the ISH probe of the target in that hybridization round, and its color indicates the fluorophore of the hybridized reporter. '0' means that there is no reporter that binds to the target ISH probe in that hybridization round, and the target should be silenced or blank in that round of imaging. For each gene, 4 reporters will sequentially bind to the 4 designated reporter landing domains of the ISH probe throughout the 16 rounds of cyclic reporter readout." He-Beechem at 43, notes at Fig. S1  He-Beechem, Figure S1 "Analysis workflow to **transform raw images to decoded RNA transcripts at subcellular resolution**. The workflow includes: 1) three-dimensional primary image processing to identify and register reporter spots, 2) **decoding of reporter spots to RNA transcripts with registered X, Y, Z spatial location**, 3) outlining of nuclei and cell boundaries with DAPI and antibodies after cyclic

		reporter readout for morphology-based cell segmentation, and 4) assigning RNA transcripts to single cells." He-Beechem at 8, at Fig. 1C (emphasis added)  He-Beechem, Fig. 1C "As an example of the final output of the analysis workflow, three identified genes (COL1A1 [yellow], IGHG1 [cyan], and KRT19 [orange]) in FFPE human lung tissue were overlayed with the segmented cells based on their registered spatial information." He-Beechem at 8, notes at Fig. 2C *See also* He-Beechem, Fig. 4 and notes

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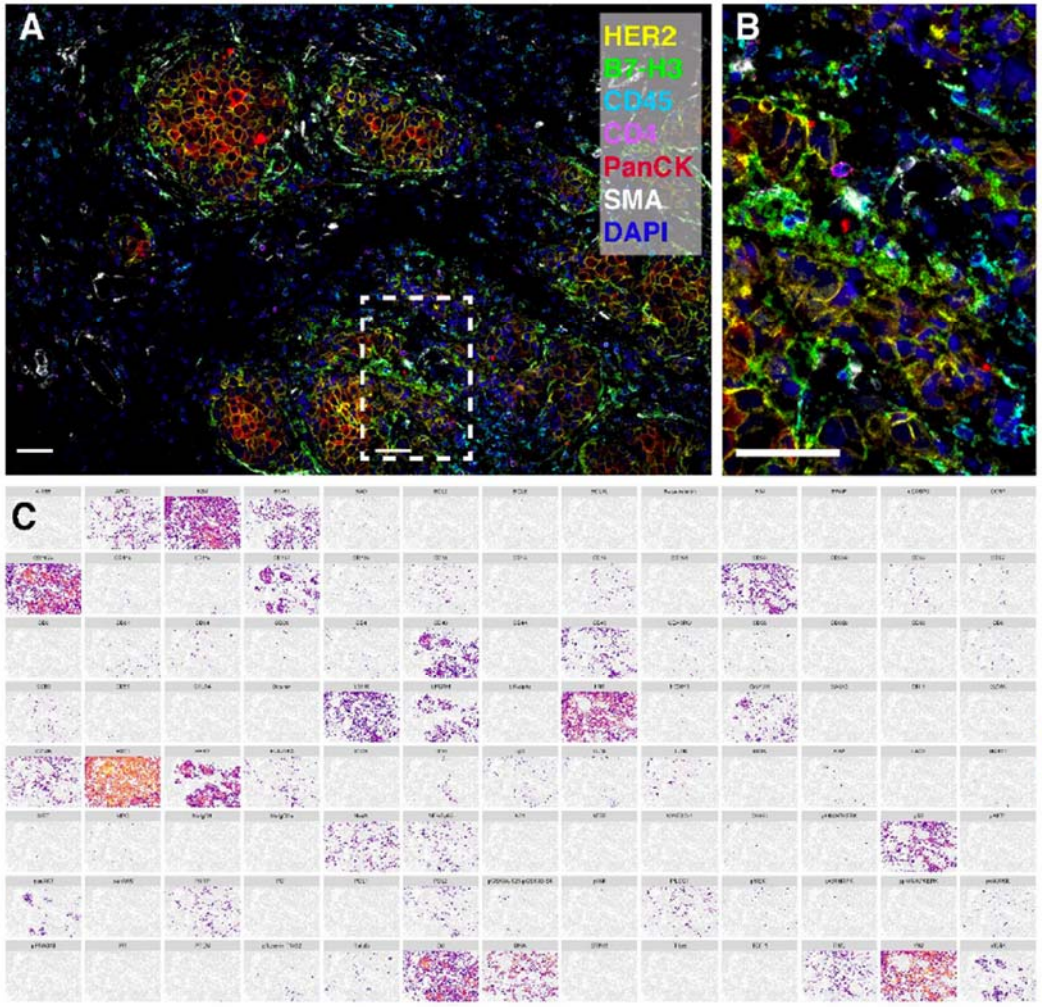
		"The **SMI encoded detection chemistry utilized for RNA can be applied to protein detection** by conjugating the oligonucleotide-readout sequences to antibodies (Figure S13). This simple expansion of SMI chemistry for antibody-based detection is enabled by the extremely small size of the oligonucleotides required for SMI 64-bit encoding; only 60 to 80 nucleotides are required to encode all of the multiplexing." He-Beechem at 19 (emphasis added)



He-Beechem, Fig. 6

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		"Spatial Subcellular Protein Analysis on SMI. A. Multi-channel overlay of six protein targets detected in a breast cancer biopsy (HER-2 positive invasive carcinoma) from a 108-plex assay (104 encoded targets and 4 morphological markers). B. Enlargement of boxed region in (A). Each decoded marker is visualized along with DAPI (blue) and the morphological markers HER2 (yellow), B7-H3 (light green), CD45 (cyan), CD4 (light purple), PanCK (red), and SMA (white). Scale bars: 50 μm. C. Single-cell expression profiles across 104-encoded protein targets in the sample shown in (A). Colored according to the log2 transformation of the sum of pixel intensities in each cell with a threshold for visualization the geometric mean of the negative controls plus 1.5 standard deviations." He-Beechem at 21-22, notes at Fig. 6